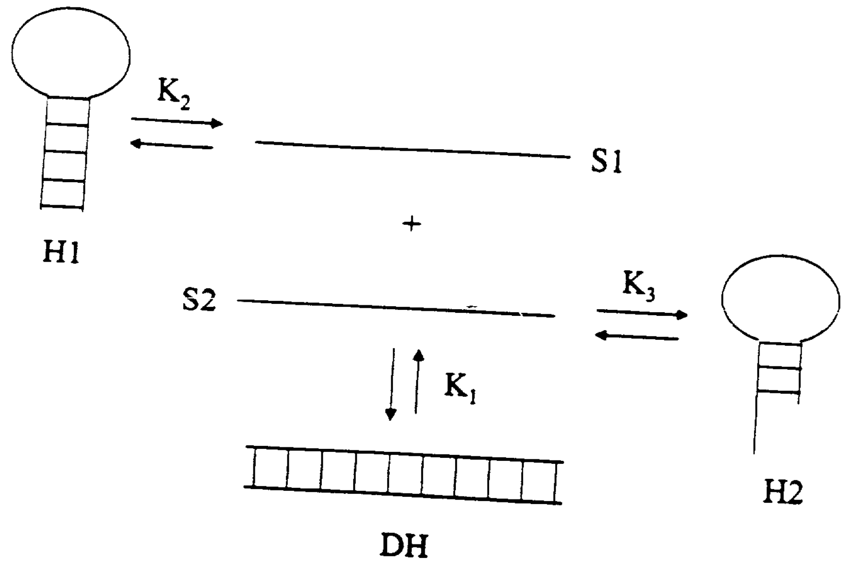


Figure 1

Module 1

Hybridization Information

Top Strand/Bottom Strand Types
DNA/DNA

Top Strand Sequence 5'-3'
cccaaaaaaaaaaaaccg

Bottom Strand Sequence 5'-3'
Use Complement
ggtttttttttttgg

Hybridization Conditions

User defined values for [Na+] and [Mg2+]

[Monovalent cation]	0.105	mol/L
[Mg^{2+}]	0	mol/L
Hybridization Temperature	37.0	°C
[Top Strand]	5e-8	mol/L
[Bottom Strand]	3e-7	mol/L

Corrections

Linear Correction for Micro Chips
($\Delta G^{o}{}_{37}$(microchip) = a x $\Delta G^{o}{}_{37}$(solution) + b)

a = 1 b = 0

Top Strand Folding Correction

$\Delta G^{o}{}_{37}$= -2.1 kcal/mol ΔH^{o}= -37.8 kcal/m

Bottom Strand Folding Correction

$\Delta G^{o}{}_{37}$= 0 kcal/mol ΔH^{o}= 0 kcal/m

Predict Thermodynamics Clear Input

Figure 2a

Module 1

Duplex sequence

5′-CCCAAAAAAAAAAACCG-3′
3′-*GGTTTTTTTTTTTGG*-5′

Experimental conditions

Hybridization type = DNA/DNA
[Top strand] = 0.5E-07 mol/L
[Bottom strand] = 0.3E-06 mol/L
Hybridization temperature = 37.0 °C

Corrections

Top strand folding:

ΔH^o = -37.8 kcal/mol
$\Delta G^o{}_{37.0}$ = -2.10 kcal/mol

Thermodynamic predictions

In 1.000 M NaCl:

ΔH^o = -119.3 kcal/mol
ΔS^o = -335.8 eu
$\Delta G^o{}_{37.0}$ = -15.14 kcal/mol
T_M = 52.9 °C

In 0.1050 M NaCl and 0.0000 M MgCl2:

ΔH^o = -119.3 kcal/mol
ΔS^o = -348.3 eu
$\Delta G^o{}_{37.0}$ = -11.29 kcal/mol
T_M = 42.2 °C

The net hybridization thermodynamics is:

$\Delta G^o{}_{37.0}$ = -8.74 kcal/mol
T_M = 34.9 °C

Note:
The net hybridization temperature is the temperature at which the concentration of duplex equals half the maximum possible concentration of duplex.
The net free energy is calculated from the net equilibrium constant at the given temperature: Knet=[Duplex]/((Ct-[Duplex])*(Cb-[Duplex])), where [Duplex] is the concentration of duplex, Ct is the initial concentration of top strand, Cb is the initial concentration of bottom strand.

Figure 2b

Module 2

Hybridization Information

Target/Primer Types

DNA/DNA ▾

Target Sequence 5'-3'

Primer length 15

Number of best primers to be displayed 2

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ▾

[Monovalent cation]	1	mol/L
$[Mg^{2+}]$	0	mol/L
Hybridization Temperature	37.0	°C
[Target]	1e-6	mol/L
[Primer]	1e-6	mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{o}{}_{37}(\text{microchip}) = a \times \Delta G^{o}{}_{37}(\text{solution}) + b)$

a = 1 b = 0

Predict Primers | Clear Input

Figure 3a

Module 2

Target sequence

5′- A C C G T T T G T A G T C C G T A C G A C A C A T A A C G G T G C A T T (

Experimental conditions

Hybridization type = DNA/DNA
[Top strand] = 0.1E-05 mol/L
[Bottom strand] = 0.1E-05 mol/L
Hybridization temperature = 37.0 °C
$[Na^+]$ = 1.0000 mol/L
$[Mg^{2+}]$ = 0.0000 mol/L

Corrections

No corrections

The 2 best primers of length 15 are:

From position 28 to 42: 5′-GGTTGCAATGCACCG -3′

ΔH^o = -132.0 kcal/mol ΔS^o = -355.6 eu $\Delta G^o_{37.0}$ = -21.71 kcal/mol T_M = 70.2 °C

From position 35 to 49: 5′-GCAGCATGGTTGCAA -3′

ΔH^o = -124.8 kcal/mol ΔS^o = -336.5 eu $\Delta G^o_{37.0}$ = -20.42 kcal/mol T_M = 68.4 °C

Figure 3b

Module 3

Hybridization Information

Target/Primer type
DNA/DNA ▾

Target 5'-3'
acgcttgaatgcagttaatgcc

Primer Sequence 5'-3' ▾
tgaatgcagt

Minimum percent stability of alternative binding sites compared to the most stable binding site: 50

Number of base pairs required to compute the solution: 5

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ▾

[Monovalent cation]	1	mol/L
[Mg^{2+}]	0	mol/L
Hybridization Temperature	37.0	°C
[Target]	1e-6	mol/L
[Primer]	1e-6	mol/L

Corrections

Linear Correction for Micro Chips
(ΔG^{o}_{37}(microchip) = a x ΔG^{o}_{37}(solution) + b)

a = 1 b = 0

Submit Clear Input

Figure 4a

Module 3

Target sequence

5′- A C G C T T G A A T G C A G T T A A T G C C -3′

Primer sequence

3′-TGACGTAAGT-5′

Experimental conditions

Hybridization type = DNA/DNA
[Top strand] = 0.1E-05 mol/L
[Bottom strand] = 0.1E-05 mol/L
Hybridization temperature = 37.0 °C
$[Na^+]$ = 1.0000 mol/L
$[Mg^{2+}]$ = 0.0000 mol/L
Number of base pairs required to compute the solution = 5

Corrections

No corrections

Best primer site

from target position 8 to position 17

```
GAATGCAGTTAA
 TGACGTAAGT
```

ΔH^o = -26.2 kcal/mol ΔS^o = -70.7 eu $\Delta G^o_{37.0}$ = -4.28 kcal/mol
T_M = -9.9 °C

Figure 4b

Hybridization Information

Target/Primer Types

DNA/DNA ▾

Target Sequence 5'-3'

Find best primer in sequence section ranging from nucleotide number:

1 to 10

Primer length 15

Number of best primer 1

Percent stability of alternative binding sites compared to the most stable binding site 50

Number of base pairs required to compute the solution 7

Hybridization Conditions

User defined values for [Na+] and [Mg2+] ▾

[Monovalent cation]	1	mol/L
$[Mg^{2+}]$	0	mol/L
Hybridization Temperature	37.0	°C
[Target]	1e-6	mol/L
[Primer]	1e-6	mol/L

Corrections

Linear Correction for Micro Chips

$(\Delta G^{o}{}_{37}(\text{microchip}) = a \times \Delta G^{o}{}_{37}(\text{solution}) + b)$

a = 1 b = 0

Submit Clear Input

Figure 5a

Target sequence

Module 5

5′-
AGGTCCATGCTTTGGAACAGCTACTTGAACCGATCATGGACACTGACGGATAAC(
-3′

Experimental conditions	Corrections
Hybridization type = DNA/DNA [Top strand] = 0.1E-05 mol/L [Bottom strand] = 0.1E-05 mol/L Hybridization temperature = 37.0 °C $[Na^+]$ = 1.0000 mol/L $[Mg^{2+}]$ = 0.0000 mol/L Number of base pairs required to compute the solution = 7 Best primer search area from position 1 to position 60	No corrections

Best primer # 1:

from target position 35 to 49
5′-TCATGGACACTGACGGA-3′
 3′-GTACCTGTGACTGCC-5′

ΔH^o = -123.1 kcal/mol ΔS^o = -331.5 eu $\Delta G^o_{37.0}$ = -20.27 kcal/mol T_M = 68.4 °C

Best primer # 2:

from target position 18 to 32
5′-ACAGCTACTTGAACCGA-3′
 3′-GTCGATGAACTTGGC-5′

ΔH^o = -125.0 kcal/mol ΔS^o = -339.6 eu $\Delta G^o_{37.0}$ = -19.67 kcal/mol T_M = 66.1 °C

Figure 5b

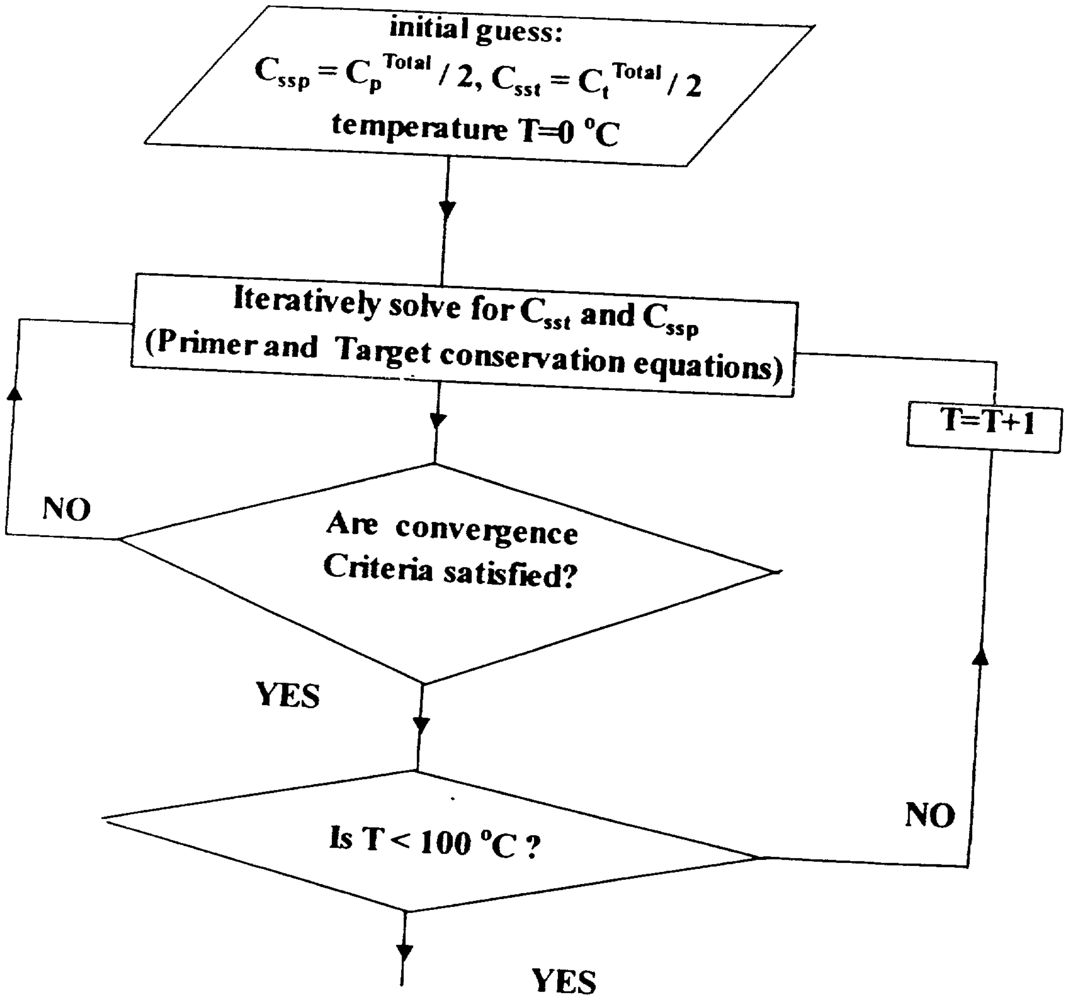


Figure 6

Multiplex PCR Design

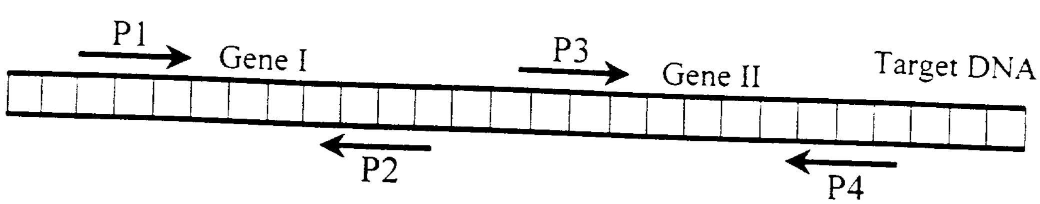


Figure 7

Prediction of Molecular Beacon Hybridization

Duplex Formation

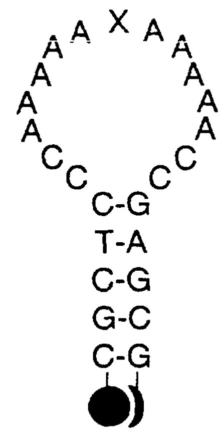
CCCAAAAAXAAAAACCG
+
GGTTTTTYTTTTTGG
⇄
CCCAAAAAXAAAAACCG
GGTTTTTYTTTTTGG

X-Y	ΔG^{o}_{37}	T_M
A-T	-13.24	47.9
A-A	-9.94	39.1
A-C	-9.03	36.0
A-G	-10.36	40.4

Beacon Folding

Random Coil Beacon ⇄

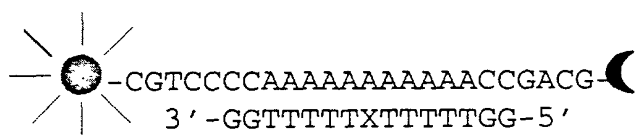
ΔG^{o}_{37} = -2.1 kcal/mol T_M = 55.2 °C



Hairpin Beacon

Net Hybridization

Hairpin Beacon
+
Target
⇄



Target	X-Y	ΔG^{o}_{37} (Effective)		T_M (Effective)	
		Exp.	Pred.	Exp.	Pred.
3'GGTTTTTTTTTTTGG5'	A-T	-10.49	-10.69	42	42.4
3'GGTTTTTATTTTTGG5'	A-A	-6.66	-7.39	27	26.8
3'GGTTTTTCTTTTTGG5'	A-C	-6.72	-6.48	23	21.1
3'GGTTTTTGTTTTTGG5'	A-G	-7.62	-7.81	28	29.5

0.105 M NaCl 0.001 M $MgCl_2$ [beacon] = 5×10^{-8} M [target] = 3×10^{-7}M

Bonnet et al. (1999), *Proc. Nat. Acad. Sci. USA 96*, 6171-6176

Figure 8

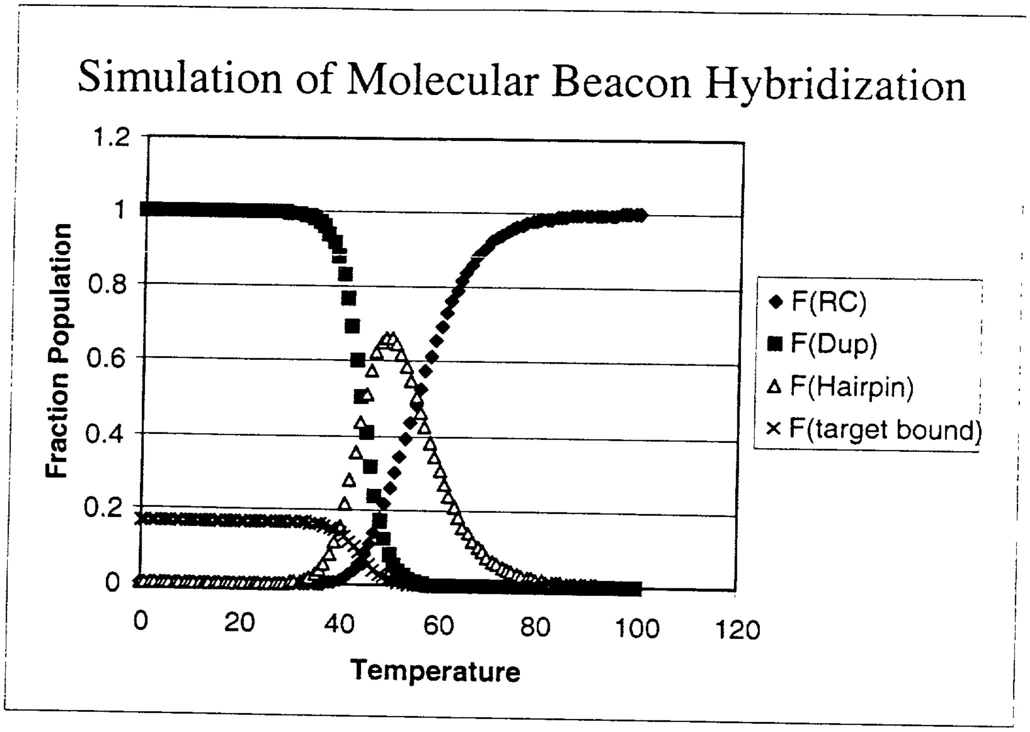
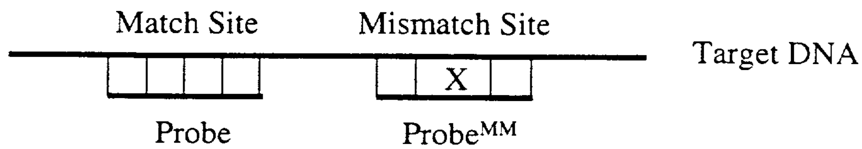

Simulation of Molecular Beacon Hybridization
1.2
1
0.8
0.6
0.4
0.2
0
Fraction Population
0
20
40
60
80
100
120
Temperature
F(RC)
F(Dup)
F(Hairpin)
F(target bound)

Figure 9

Match vs. Mismatch Hybridization



Equilibria

Match Hybrid	$T + P \rightleftharpoons T\text{-}P$		$K_M = \dfrac{[T\text{-}P]}{[T]\,[P]}$
Mismatch Hybrid	$T + P \rightleftharpoons T\text{-}P^{MM}$		$K_{MM} = \dfrac{[T\text{-}P^{MM}]}{[T]\,[P]}$
Double Hybrid	$T + 2P \rightleftharpoons T\text{-}P\text{-}P^{MM}$		$K_{DH} = \dfrac{[T\text{-}P\text{-}P^{MM}]}{[T]\,[P]^2}$

- Given C_{Target}[total], C_{Probe}[total]and the 3 equilibrium constants above, it is trivial to solve for the concentrations of all species
- Since $\Delta G°_{37}$ and $\Delta H°$ are known, calculate K's at all temperatures
- Simulate hybridization at all temperatures - optimize specificity
- More complex model would also include single-strand folding equilibria

Figure10

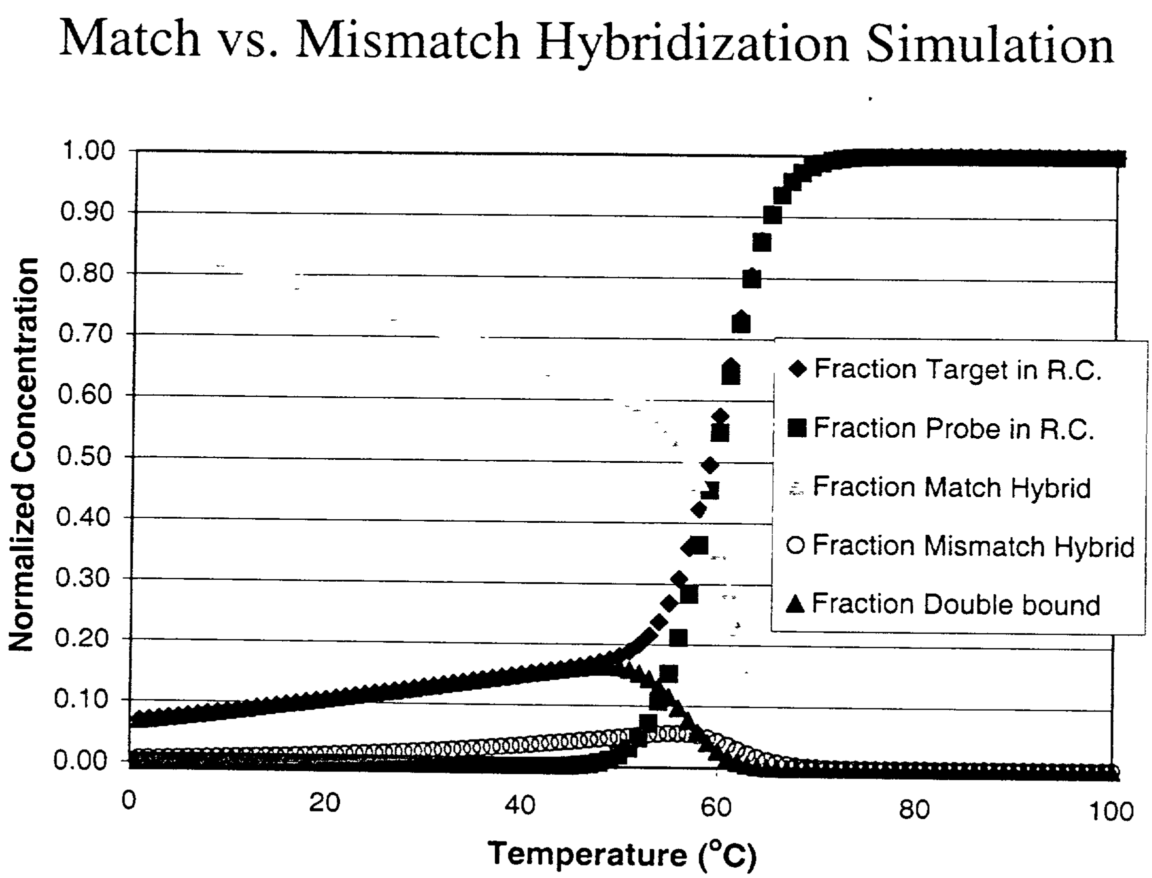

Match vs. Mismatch Hybridization Simulation
Normalized Concentration
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
20
40
60
80
100
Temperature (°C)
Fraction Target in R.C.
Fraction Probe in R.C.
Fraction Match Hybrid
Fraction Mismatch Hybrid
Fraction Double bound

Figure11

SCANNED, #12

CONCENTRATION CALCULATIONS

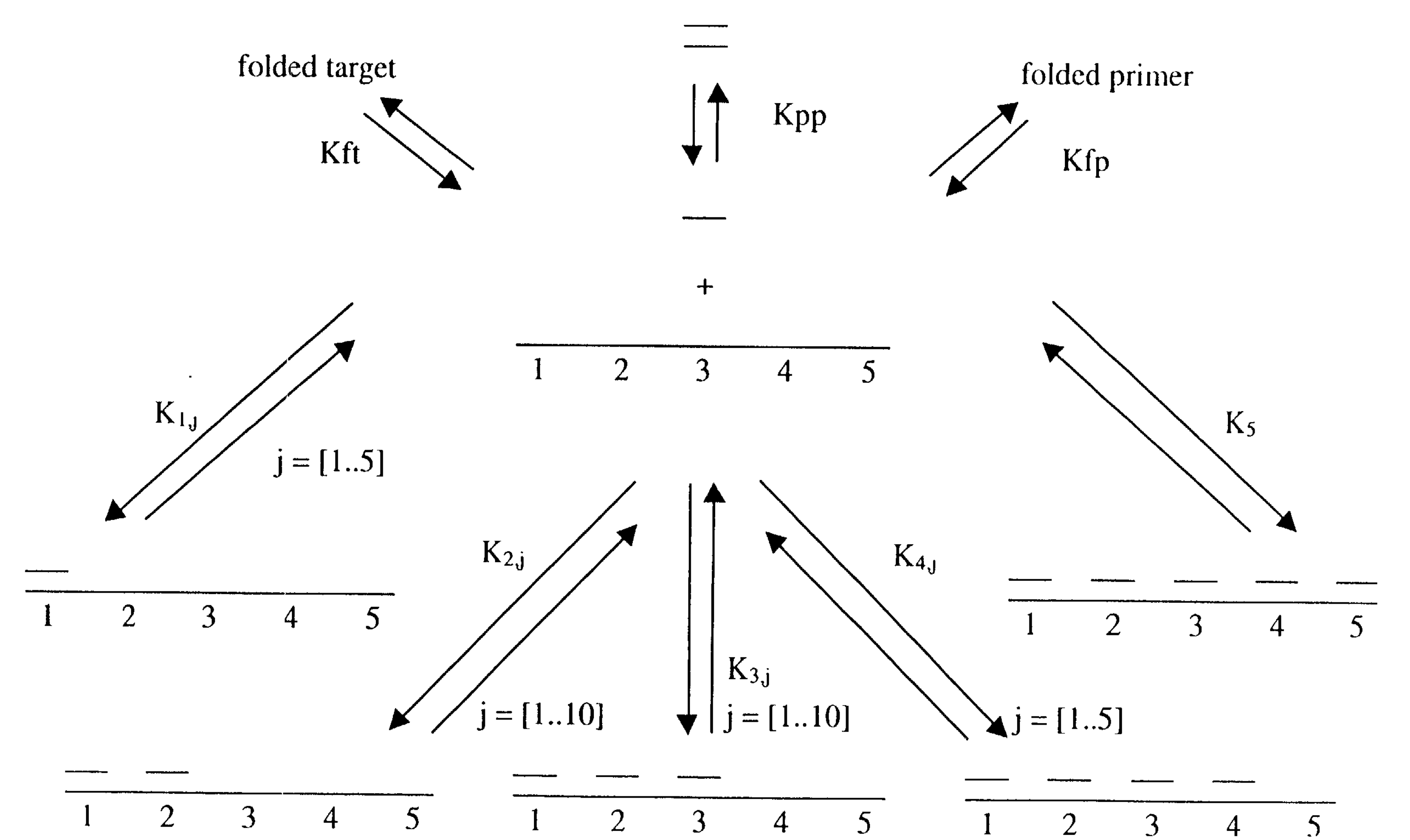


Figure12

Title: Method and System For Predicting Nucleic And Acid Hybridization Thermodynamics And Computer-Readable Storage Medium For Use Therein
First Named Inventor: John SantaLucia, Jr., et al.
Application Serial No.: / Atty. Docket No.: WSU 0192 PUSP

PRIMER AND TARGET CONSERVATION EQUATIONS

$$C_{sst} = C_t^{Total} / \{1 + K_{ft} + 2 \times K_{tt} \times C_{sst}$$

$$+C_{ssp} \times (K_1 + K_2 + K_3 + K_4 + K_5)$$

$$+C_{ssp}^2 \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5})$$

$$+C_{ssp}^3 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5} + K_{3,4,5} + K_{2,4,5})$$

$$+C_{ssp}^4 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5})$$

$$+ C_{ssp}^5 \times K_{1,2,3,4,5}\}$$

$$C_{ssp} = C_p^{Total} / (1 + K_{fp} + 2 \times K_{pp} \times C_{ssp}$$

$$+ C_{sst} \times (K_1 + K_2 + K_3 + K_4 + K_5)$$

$$+2 \times C_{sst} \times C_{ssp} \times (K_{1,2} + K_{1,3} + K_{1,4} + K_{1,5} + K_{2,3} + K_{2,4} + K_{2,5} + K_{3,4} + K_{3,5} + K_{4,5})$$

$$+3 \times C_{sst} \times C_{ssp}^2 \times (K_{1,2,3} + K_{1,2,4} + K_{1,2,5} + K_{1,3,4} + K_{1,3,5} + K_{1,4,5} + K_{2,3,4} + K_{2,3,5}$$

$$+ K_{3,4,5} + K_{2,4,5})$$

$$+ 4 \times C_{sst} \times C_{ssp}^3 \times (K_{1,2,3,4} + K_{1,2,3,5} + K_{2,3,4,5} + K_{1,3,4,5} + K_{1,2,4,5})$$

$$+5 \times C_{sst} \times C_{ssp}^4 \times K_{1,2,3,4,5})$$

Figure13